

09/509188

412 Rec'd PCT/PTO 23 MAR 2000



FORM PTO-1390 (Modified) (REV 5-93)		U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		065691/0184	
		U.S. APPLICATION NO (If known, see 37 CFR 1.5) Unassigned	
INTERNATIONAL APPLICATION NO. PCT/FR98/02042	INTERNATIONAL FILING DATE September 23, 1998	PRIORITY DATE CLAIMED September 23, 1997	
TITLE OF INVENTION MICROSPORE-SPECIFIC PROMOTER AND METHOD FOR PRODUCING HYBRID PLANTS			
APPLICANT(S) FOR DO/EO/US Jan DROUAUD, Agnes FOURGOUX, Georges PELLETIER and Philippe GUERCHE			
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:			
<p>1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.</p> <p>2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.</p> <p>3. <input type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).</p> <p>4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.</p> <p>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)) <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau). <input checked="" type="checkbox"/> has been transmitted by the International Bureau. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US)</p> <p>6. <input checked="" type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)).</p> <p>7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau). <input type="checkbox"/> have been transmitted by the International Bureau. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. <input checked="" type="checkbox"/> have not been made and will not be made.</p> <p>8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</p> <p>9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).</p> <p>10. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</p>			
Items 11. to 16. below concern other document(s) or information included:			
<p>11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</p> <p>12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</p> <p>13. <input type="checkbox"/> A FIRST preliminary amendment. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.</p> <p>14. <input type="checkbox"/> A substitute specification.</p> <p>15. <input type="checkbox"/> A change of power of attorney and/or address letter.</p> <p>16. <input checked="" type="checkbox"/> Other items or information: Copy of Sequence Listing (4 pgs)</p>			

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U.S. APPLICATION NO. if known, See 37 CFR 1.5 Unassigned	097509188	INTERNATIONAL APPLICATION NO PCT/FR98/02042	ATTORNEY'S DOCKET NUMBER 065691/0184			
17. <input checked="" type="checkbox"/> The following fees are submitted:			CALCULATIONS PTO USE ONLY			
Basic National Fee (37 CFR 1.492(a)(1)-(5): Search Report has been prepared by the EPO or JPO \$840.00						
International preliminary examination fee paid to USPTO (37 CFR 1.482) \$670.00						
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) \$690.00						
Neither international preliminary examination fee (37 CFR 1.482) nor International search fee (37 CFR 1.445(a)(2)) paid to USPTO \$970.00						
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) \$96.00						
ENTER APPROPRIATE BASIC FEE AMOUNT =			\$840.00			
Surcharge of \$130.00 for furnishing the oath or declaration later than 20 Months from the earliest claimed priority date (37 CFR 1.492(e))						
Claims	Number Filed	Included in Basic Fee	Extra Claims	Rate		
Total Claims	34	-	20	= 14	x \$18.00	\$252.00
Independent Claims	3	-	3	= 0	x \$78.00	\$0.00
Multiple dependent claim(s) (if applicable)					\$260.00	\$260.00
TOTAL OF ABOVE CALCULATIONS =			\$1352.00			
Reduction by ½ for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).						\$0.00
SUBTOTAL =			\$1352.00			
Processing fee of \$130.00 for furnishing English translation later the 20 months from the earliest claimed priority date (37 CFR 1.492(f)). +						
TOTAL NATIONAL FEE =			\$1352.00			
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +						
TOTAL FEES ENCLOSED =			\$1352.00			
			Amount to be: refunded \$			
			charged \$			
a. <input checked="" type="checkbox"/>	A check in the amount of \$1352.00 to cover the above fees is enclosed.					
b. <input type="checkbox"/>	Please charge my Deposit Account No. <u>19-0741</u> in the amount of \$1352.00 to the above fees. A duplicate copy of this sheet is enclosed.					
c. <input checked="" type="checkbox"/>	The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>19-0741</u> . A duplicate copy of this sheet is enclosed.					
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.						
SEND ALL CORRESPONDENCE TO:						
Foley & Lardner Washington Harbour 3000 K Street, N.W., Suite 500 Washington, D.C. 20007-5109						
_____ SIGNATURE NAME PATRICIA D. GRANADOS						
REGISTRATION NUMBER 33,683						

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No. :

U.S. National Serial No. :

Filed :

PCT International Application No. : PCT/FR98/02042

VERIFICATION OF A TRANSLATION

I, the below named translator, hereby declare that:

My name and post office address are as stated below;

That I am knowledgeable in the French language in which the below identified international application was filed, and that, to the best of my knowledge and belief, the English translation of the international application No. PCT/FR98/02042 is a true and complete translation of the above identified international application as filed.

I hereby declare that all the statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent application issued thereon.

Date: 22 February 2000



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MICROSPORE-SPECIFIC PROMOTER AND METHOD FOR PRODUCING
HYBRID PLANTS

The present invention concerns in particular a
5 microspore-specific promoter and a method for producing
hybrid plants.

The microspore corresponds to a precise stage
in the development of the male gamete in higher plants.
Male gametogenesis takes place in a specialized organ,
10 the anther, and comprises *sensu stricto* the
differentiation of diploid cells into haploid pollen
grains. Each diploid cell, called a sporogenic cell,
undergoes meiosis to produce four haploid microspores
which subsequently differentiate to give mature pollen
15 grains.

Knowing the molecular factors which control the
development of the microspore, and how to manipulate
them, is a considerable asset not only from a
fundamental research point of view but also from a
20 plant-improvement point of view. This is because this
knowledge enables the production of pollen grains, and
consequently the reproduction of the plant, to be
controlled.

Such control proceeds via the production of
25 plants with one of their gametes totally sterile so as
to prevent self-fertilization.

So far, male sterility of plants, which is less
complex than female sterility, has been widely studied
but necessitates the use of genetic systems which are
30 relatively laborious to implement for commercial
production of hybrid seeds. One type of male sterility
which is highly used is cytoplasmic male sterility
which consists in producing:

35 - a female line whose sterile-male character-
istic is transmitted through the cytoplasm;
such a cytoplasm is called a "male-sterility-
inducing cytoplasm"; these "inducing
cytoplasms" are, for a given species, in
general discovered in the wild, or sometimes

observed in plants which result from interspecific crosses (cross-fertilization, protoplast fusion, etc.).

- 5 - a "sterility-maintaining" line whose cytoplasm is normal,
- and
- a fertility-restoring line if the seeds and/or the fruit of the hybrid plant are harvested.

10 In the female line (carrier of the sterility-inducing cytoplasm) all the pollen grains are killed. To multiply and improve this line it is therefore necessary to have a line which carries neither the inducing cytoplasm (which thus produces pollen grains) 15 nor the restoration gene. This line is termed "sterility-maintaining" because crossing with the female line gives an entirely female lineage.

20 Restoration of the fertility is carried out in the hybrid by crossing the female parent (carrying the sterile male cytoplasm) with the parent comprising a nuclear restoration gene (the restoring line), this cross enabling the production of fertile hybrid plants which will produce seeds by self-fertilization.

25 In the case of sporophytic nuclear sterility, systems have been described, for example, which make it possible to kill the mother cells of the microspores by means of an RNase, and, consequently, to obtain plants lacking in the male gametes. The fertility is restored when the line which no longer produces male gametes is 30 crossed with another line carrying an inhibitor of the RNase, the seeds resulting from this cross comprising both the cytotoxic gene and its inhibitor.

As for the present invention, it proposes to 35 produce plants with gametophytic male sterility, which are incapable of producing pollen grains. It consists in using a promoter region which controls the expression, specifically in the microspores, of a gene encoding a cytotoxic molecule, while also having a

means permitting the controlled inhibition of this toxicity, in order to obtain a line of homozygous progenitor plants which are totally sterile as regards their male gametes, and then to obtain fertile hybrid 5 plants (which produce one viable pollen grain in two), thus capable of producing seeds, without having to resort to using a fertility restoration gene.

So far, a single gene which is expressed specifically in the microspore has been described, in 10 tobacco (Oldenhof et al., 1996). This gene does not have any homology with the Brassicaceae as results from a Southern Blot experiment on the genomic DNA of *Brassica oleracea* (data not shown).

A subject of the present invention is therefore 15 a nucleotide sequence for which it has been demonstrated that the corresponding gene is expressed specifically in the microspore; this nucleotide sequence corresponds to SEQ ID No. 3.

Consequently, a subject of the present 20 invention is a nucleotide sequence corresponding to all or part:

- a) of the sequence according to SEQ ID No. 3,
or
- b) of a sequence which hybridizes to the
25 sequence according to a), or
- c) of a sequence which has at least 80%
homology with a) or b).

In the context of the present invention, the most valuable part of this nucleotide sequence is the 30 promoter region which is defined as being the sequence preceding (on the 5' side) the translation start codon (ATG). However, at the current stage of knowledge about the nucleotide sequence according to SEQ ID No. 3, three ATGs have been shown: one at position 1965, 35 another at position 2085 and a third at position 2112. It would appear that the functional ATG is the one situated at position 2085. This is not confirmed however; it is the reason why the largest envisagable

promoter region concerning SEQ ID No. 3 stretches from nucleotide 1 to nucleotide 2111, and preferably from nucleotide 1 to nucleotide 2084.

This promoter region thus precedes, in the 5 natural state, a coding (orf) sequence which is expressed specifically in the microspores, and in the case where this orf is replaced (by genetic manipulation) by another orf whose product is a cytotoxic molecule, the latter is capable of destroying 10 only said microspores.

A subject of the invention is therefore also cellular expression vectors, comprising a promoter sequence such as that described above, placed upstream of a DNA sequence encoding a cytotoxic product.

15 Advantageously, the cytotoxic product in question is a protease. Specifically, when the protease is expressed specifically in the microspores, it destroys all the proteins thereof, as a result of which the microspore cannot survive. Preferably, the protease 20 is a subtilisin, and in particular the BPN' subtilisin from *Bacillus amyloliquefaciens*. This BPN' subtilisin is part of the family of subtilisins which are found in many organisms and which are proteases known to cleave proteins at the level of serines.

25 It involves, therefore, introducing a vector in accordance with the invention into a bacterial strain capable of carrying out the transformation of plant cells, such as *Agrobacterium tumefaciens*. This may in particular be carried out by the method of infiltration 30 of *Arabidopsis thaliana* plants described by Bechtold et al., 1993. This technique consists in introducing the bacterium into the cells of the floral scapes by infiltration under vacuum. The plants are then bedded out under glass and their seeds harvested. About one 35 seed in a thousand gives rise to plants of which all the cells carry the transgene. The transformation of other plants, and in particular of rape, may be carried out through *Agrobacterium tumefaciens* and/or

Agrobacterium rhizogenes with the aid of various techniques, now conventional (transformation of foliar disks, of hypocotyls, of floral scapes etc.) which combine a phase of coculture of the bacterium with the 5 plant tissues, followed by the selection and by the regeneration of the transformed cells into whole plants. Other transformation techniques do not use this bacterium, but make it possible to transfer the cloned gene directly into cells or tissues (electroporation, 10 particle gun etc.), and to select and obtain transformed plants (review by Siemens and Schieder).

A subject of the present invention is also the cells of plants transformed with a vector in accordance with the invention and plants comprising said cells.

15 A subject of the invention is also plants with gametophytic male sterility with inducible fertility, comprising a gene encoding a male-gamete-specific cytotoxic product.

As indicated above, the present invention thus 20 enables the production of plants with gametophytic male sterility which inhibits any production of pollen grains. However, these plants, which are homozygous as regards their male sterility, may be obtained only after self-fertilization of plants which have 25 previously been transformed with a vector in accordance with the invention, i.e. which are hemizygous as regards their male sterility and in which the fertility of the pollen grains carrying the gametophytic sterility has been provisionally restored, so as to 30 allow them to carry out self-fertilization.

One means of producing plants which are homozygous for this gene would be to use gynogenesis, a technique which consists in regenerating doubled haploid plants from ovule or ovary culture. It 35 involves, in this case, obtaining the formation of a homozygous diploid plant from a female haploid gamete. Gynogenesis is applicable to a certain number of plant species, but production of a large number of plants

which are homozygous for the transgene in question is not envisagable by this technique, because it is tricky to use and its efficacy most often remains very poor.

The present invention also concerns a method
5 for producing plants with gametophytic male sterility with inducible fertility, comprising:

- the insertion into plants of line A of a gene whose expression product is cytotoxic for the microspores, and
- 10 - the production of plants which do not produce male gametes.

More particularly, the method for producing plants with gametophytic male sterility with inducible fertility in accordance with the invention comprises
15 the steps of:

- a) transformation of plants of a line A with a vector in accordance with the invention,
- b) induction of the fertility of the plants obtained in a) by inhibition of the cytotoxicity of the product,
- 20 c) self-fertilization of the fertile plants obtained in b),
- d) selection of the plants which do not produce male gametes, derived from c),
- 25 e) multiplication of the plants obtained in d) by repeating steps b) and c).

Thus, in step a) of the method above, a line A is transformed with a vector in accordance with the invention, i.e. comprising a microspore-specific promoter sequence placed upstream of a gene encoding a cytotoxic product. The plants resulting from this transformation all comprise the DNA in question whose gene is expressed only in the microspores. However, at this stage, the plant being diploid at the time of the transformation, it becomes heterozygous as regards its male sterility and is therefore capable, after transformation, of giving rise to microspores of which

the transgene, are thus destroyed by the cytotoxic product). A 50% production of the pollen is more than enough to give rise to seeds having the qualities of each of the crossed lines which it is specifically
5 desired to combine.

The present invention thus also concerns a method for producing hybrid plants, characterized in that it comprises crossing plants of line A, which have gametophytic male sterility as described above, with
10 plants of line B of agronomic value. It also concerns the seeds derived from the hybrid plants thus obtained.

Advantageously, the plants in accordance with the invention belong to the Brassicaceae family; preferably, they are rape.

15 In addition, it should be pointed out that the promoter region in accordance with the invention may also be used in strategies of gene inactivation by utilization of mobile elements such as transposons and retrotransposons.

20 Specifically, this may be carried out with the aim of isolating plants which have a stable mutant genotype, and isolating a very large number of different, independent mutants.

It involves creating a chimeric sequence
25 consisting of a promoter region in accordance with the invention and of the sequence, all or in part, of a mobile element. The expression of this mobile element, which is reduced to the phase of development of the microspore, should make it possible to induce some
30 mutations into the genome of the pollen grains of the transformed plant. It is thus possible, in the lineage obtained from these pollen grains, to isolate individuals which no longer carry the transgene, but merely one or more mutations derived from transposition
35 phenomena. The principle is to bring about, using the abovementioned promoter region, activation of the transposition of these mobile elements for a very short time (microsporogenesis) in a multitude of gametic

cells and to eliminate in the following generation the plants which carry the transgene (i.e. the promoter region + the sequence which allows the activation of the transposition) so that the cycle does not start up again. It then involves investigating, in the lineage, and by various techniques, the plants for which the mobile elements have caused mutations by inserting themselves into genes. The study of these plants would make it possible, in particular, to understand the function of the mutated gene.

Among the mobile elements which can be used in this way, mention may be made of the retrotransposons of the type Tnt1, Tto1, Tnp-2, Tos10-17, Bsl, BARE-1, Ta-1, etc., or the transposons of the type Ac/Ds, Spm, Mu, etc.

Figure 1 illustrates the alignment of the sequences of the two cDNAs M3 (SEQ ID No. 1) and M3.21 (SEQ ID No. 2) derived from the screenings of the *Brassica napus* cv.Brutor microspore cDNA library. The start (ATG) and stop (TGA) codons of the putative coding sequence are underlined.

Figure 2 gives the nucleotide sequence of the clone BnM3.4 (SEQ ID No. 3) from which the M3 cDNA is thought to be derived. The ATG in bold (position 2085) is the one which has the highest probability of being the functional ATG. The ATG underlined in position 2112 is the one present in the M3.21 cDNA sequence. The ATG underlined in position 1965 is the first ATG encountered. The sequence preceding these ATGs is, consequently, taken to be the promoter region of the BnM3.4 gene.

Figure 3 illustrates the Northern Blot hybridization with the P^{32} -labeled M3 probe on total RNAs (10 μ g per well) extracted from different rape tissues. A: buds of 0-2 mm (meiocytes); B: buds of 2-3 mm (mononucleated microspores); C: buds of 3-4 mm (binucleated microspores); D: buds greater than 4 mm (mature pollen grains); E: rape sepals; F: rape

pistils; G: buds of sterile male rape; H: full buds of rape.

Figure 4 illustrates the preparation of the 7152 bp pJD51 plasmid from the 5135 bp pAF1 plasmid (plasmid of origin: pBluescript SK-PROMEGA) and from the 5458 bp pBnB2 plasmid (plasmid of origin pBS SK-PROMEGA).

Figure 5 illustrates the preparation of the 19670 bp pJD101 plasmid from the 15400 bp pEC2 plasmid which is derived from the pDHB 321.1 plasmid (D. Bouchez, personal communication) and from the pJD51 plasmid (cf. Figure 2).

Figure 6 represents a scheme of selection of hybrid varieties of a plant (rape for example) which calls upon a system of gametophytic male sterility with induction of the fertility. SMGfi: gametophytic male sterility with inducible fertility; Induction F: induction of the fertility; AF: self-fertilization.

The invention is not limited to the sole description above; it will be better understood in the light of the following examples, which are given, however, purely as illustrations.

EXAMPLE 1: Demonstration of a microspore-specific promoter

The first step consisted in producing complementary DNA (cDNA) clones expressed specifically in the microspore of rape. For this, cDNAs were synthesized from rape microspore messenger RNAs (mRNA). In parallel, cDNAs were synthesized from floral bud mRNA from sterile male rape. The cDNAs coming from said floral buds were subtracted from the cDNAs derived from the mRNAs expressed in the microspore of rape. The molecules resulting from this subtraction were used in an experiment of differential hybridization of a microspore cDNA library, according to a technique similar to that presented by Atanassov et al. (1996).

One of these isolated clones, the M3 cDNA (SEQ ID No. 1), proved to be the representative of an mRNA which is specifically expressed in the microspore of rape. Another cDNA, named M3.21 (SEQ ID No. 2) was 5 found by screening the library with the M3 cDNA. The sequences of these two cDNAs show 89% identity (Figure 1); they are clearly derived from a family of very close genes, which are expressed specifically in the microspore.

10 The M3 cDNA clone was used as a probe to screen a rape genomic DNA library sold by CLONTECH Laboratories, Inc., 4030 Fabian Way, Palo Alto, CA 94303-4607, USA; two clones (BnM3.4 and BnM3.2) corresponding to two different genes were isolated. The 15 M3 cDNA is thought to be derived from the BnM3.4 (SEQ ID No. 3) gene, because this gene carries an orf which is identical to the M3 cDNA (Figure 2). This gene has no intron. Sufficient experimental results lead to the thought that the M3.21 cDNA is not derived from the 20 second isolated gene (BnM3.2), which indeed carries a region corresponding to the M3.21 cDNA sequence, but to a third gene, which is very close to the BnM3.2 gene.

The promoter region of this gene is defined as being the sequence immediately upstream of the 25 translation start codon (ATG).

EXAMPLE 2: Verification of the specificity of the promoter of the BnM3.4 gene

A/ Northern Blot

30 A Northern Blot analysis was carried out with 10 µg of total RNA from sepals, pistil, whole buds, buds from sterile male plants, meiocytes, microspores, binucleated pollen grains and trinucleated pollen grains, hybridized with the M3 cDNA. A band of 1 kb corresponds to the transcript of the BnM3.4 gene, and 35 also to the M3.21 transcript, since they are very close sequences. These transcripts are present uniquely in the first two stages of male gametogenesis, whose

products are difficult to isolate perfectly experimentally (Figure 3).

The proteins deduced from these two cDNA clones are evidently very close and are rich in glycine and proline. They are identical to strictly no other protein in the databanks, but are certainly involved in the formation of the wall.

B/ Transformation with a chimeric gene

Different chimeric genes (i.e. consisting of the sequence encoding a known gene, preceded by the promoter region in accordance with the invention) were constructed in order to study the spatio-temporal specificity of the BnM3.4 promoter.

Figure 4 shows the construction of a bacterial vector pJD51, which combines a fragment of the BnM3.4 promoter with the sequence encoding the β -glucuronidase gene. The pAF1 plasmid containing the sequence encoding β -glucuronidase and the transcription termination sequence of the NOS gene from *Agrobacterium tumefaciens*, was digested with the enzymes BamHI and ClaI. The pBnB2 plasmid contains a 6 kb BamH1-BamH1 fragment derived from the BnM3.4 genomic DNA clone, and in which the BnM3.4 gene is present. A fragment corresponding to the largest promoter region possible given the restriction sites (2056 bp) was isolated from the pBnB2 plasmid by a BamHI-NspV digestion, and inserted between the BamHI and ClaI (compatible with NspV) sites of the pAF1 plasmid.

The chimeric gene thus constructed was isolated by a NotI digestion of the pJD51 plasmid, so as to be cloned into a binary plasmid from *Agrobacterium tumefaciens*: pEC2 opened by the enzyme NotI (Figure 5).

The pJD101 binary plasmid containing the chimeric gene was introduced into the C58C1 strain (pMP90) of *Agrobacterium tumefaciens* (Koncz et al. 1986) by electroporation, and the transformants possessing pJD101 were selected on a medium containing kanamycin. One of these *Agrobacterium* transformants was

used to transform *Arabidopsis thaliana* (Wassilevskja ecotype) by the method of infiltration of the floral scapes described by Bechtold et al., 1993. The transformed plants are selected using their resistance 5 to phosphinothrycin, which is conferred by a resistance gene jointly inserted into the T-DNA.

Among these plants, certain show expression of the β -glucuronidase specifically in the microspores (demonstrated by a blue coloration when a 10 β -glucuronidase-specific substrate, X-Glu, is added). No coloration is present in the adjacent tissues of the anther, nor in the somatic tissues of the plant. In a transformed plant which is hemizygous for the chimeric gene, half the microspores produced are blue, because 15 only they contain the chimeric gene.

The specificity of expression conferred by this 2 kb promoter sequence is indeed restricted, within the limits of the sensitivity of the technique, to a single cell type, and from the microspore stage.

REFERENCES

Atanassov I et al. (1996) Plant Science 118, 185-194

5

Bechtold N. et al. (1993) Comptes-Rendus de l'Académie
des Sciences 316, 1194-1199

10

Koncz et al. 1986) Molecular General Genetics 204,
383-396

15 Mariani et al. Nature 347 (1990) 737-741

Oldenhof M.T. et al. (1996) Plant Molecular Biology
31, 213-225

20

Siemens and Schieder 1996. Plant Tissue Culture and
Biotechnology, 2, 66-75

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PCT/FR98/02042

- 1 -

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: INRA (INSTITUT NATIONAL DE
LA RECHERCHE AGRONOMIQUE)
(B) STREET: 147 RUE DE L'UNIVERSITE
(C) CITY: PARIS
(E) COUNTRY: FRANCE
(F) POSTAL CODE: 75007

(ii) TITLE OF THE INVENTION: Microspore-specific
promoter and method for producing hybrid plants

(iii) NUMBER OF SEQUENCES: 3

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version
#1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 497 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: M3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

TTTGGATCTT TCCATGACCC CTTCTTGACC GGCTATGTCA AGCTACATTG CTCCACCGTT	60
GTTGGATCTA CTTCACCTCC TCCTTCACAG GCTCCTTTAC ATGCTCCTTC TTACAGGGCT	120
CCTTCACATG CTCCTTCACA TGCTCCTTCA CAGGCTCCTT TAAATGCTCT TTTAAATGCT	180
CCTTTACATG CTCCTTACA TGCTCCTTCA CAGGCCCTT CACAGGGCCC TTCACAGGCC	240
CCTTTACATG CTCCTTACT GCCCCCTTCG CAGGCTCCTT CACCGGCTCA GTGATTTAGC	300
TATTTGATAG AATTACTCAA <u>GTAATGATGC</u> CCTAGGGACT TTGAGTTTT CTCGTGTTT	360

AAAGTTTGT GTTTATTTG AGAAAACCGT CTTGGATTT TAACTCACT TTGATTTTT	420
CCCTTATACA ATTTAAATTT AGAGTTTACT TATTAATTT ATAAATTAGA TGGTACTAAG	480
TTTTTATCAT AATAAAA	497

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 674 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: M3.21

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

TCTTGCTATG ATTTCTTCA TAAGATGTGT CACATCCAAA GTCACAGCAA CAGAACTAGA	60
GTCATCAACT AACCAAGAGC TCTTCCTATC GCGGCACTTG CCTCGCTTTC ACCCCAAGCC	120
ACATTGGCCG TTCTGTGGCT CCGGAAAAGC CTTCCCTGCA GGCCACTTCC GACCAACTCC	180
GTTCCATCTG CCACAGGAAG TCACCAAGATG CTTGTCCGAC AAGAAGGAGG TAGGTACATG	240
TTTGATGAT ATCGTTGAGA CTTTCTTCAC CAGGAAAGCC GTTATTGGAT CGGAATGTTG	300
CGCCGCGATC AAGAAGATGA ACAAAAGATTG TGAGAAGACC GTCTTGGAT CTTCCATGA	360
CCCCCTCTTG ACAGGCTATG TCAAACATACA TTGCTCCACC GTTGTGGAT CTACTTCACC	420
TCCTCCTTCA CATGCTCCTT CACAGGCTCC TTTACATGCT CCTTCACAGG CTCCCTTACA	480
TGCCCTCTCA CAGGCTCCTT TACTGCCCTT TTCACAGCCT CTCCCACCGG CTCAGTGATT	540
TTAGCTATTT GTTAGAATTA TTCAAGTGT GATGTCTAG GGAGTTTAG GTTTTCTTG	600
TTTTAAAATT TTGTGTTAT TTTGAGAAAA CCGTCTTGG ATCTTAACCT CACTTTGATT	660
TTTCCTTAT ACAA	674

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2853 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: BnM3.4

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GGATCCCACA AAGAAAACCG AAGAAGCAAA TGTTTCCTAC CTTCATAAAT ATATATTTGT	60
TTCAGCCTCA TCAATGTACA AACAAATCCTT TAGCTCAATG GTATAAAATGT TGTTGTTTAG	120
ATTTCAATAA CCCGGGTTCG AGTCATAGAC TTGACACTT TTCACACTT TTAAAAGTGG	180
AACGCACATA TCGCTGACGT GTCGCATCAG GAGTGATCCA ACTGCTCTAT TATAATGTAG	240
ATTTAAAAGT GGAACCCACG TATCGCTGAC GTGTCGCATC AGGAGTGATG CAACTGCCAT	300
ATTATAACGT AGATTTGACG TTATTCCTT TTAAATCTTA ATAATAATAC CAGNGCTTT	360
ACTTATTAAT TTTGNGCATN GTTATCATGG TTTATGCNCT CTTTTTTTT GANCCGTTGA	420
TTGGTTTATG CTTATTTGAA TGTNGCCNAC GTAAGAAATG AAGAACAAATT TATATTTGGA	480
GAAAATATAA TTTAATATGT TCAATATATA GAGAAAATAT TATNCCTTGA TGTTACTGTA	540
TGGATGCGAG TAGAAGATCT TTGAATAATA TTTGAGAACT TGCCTTTCT CAAAAAGTAA	600
AATATTTGAT ATGTAACCTA AGTTAACACA TGAAAATTAA AAAAAAATTA AATCAAATA	660
GAAAAAAACTG ATAGTGATCT ACCCTTCAAC GTTTGAACT TATTCTGGT TCACCCCCCTA	720
AACCTCTAAG TTCACCAAAAC AATAAAATT TATTATTGCA TATTCTATAT CTTTAGAAA	780
GTGAAACAAA ATATTATCAA GTTATATTAT GTTTTCAAA TAAAAGATA AAAATAAAT	840
AAAAAATAAT AGTAGTTACA AAAAAAAAATTAATATT TTACCAGCGT CANAAAACAC	900
TAAAACCTAA ACCCTAAATA TTAAACTTTT AGGTAAACCC TAAACCTTG GATAAATCTT	960
AAACATTAAA CATTAAAACA CTAAACCTA AATCCTAAC TCTAAACCT TAAGTGTAA	1020
AATGTTTAGT GTTTTGATT TATAGTTAG GATTATCCA AAGGTTAAG GTTTACCCAA	1080
GAGTTTATGG TTTAGGGATT ATGACTTAGG ATTTAGTGTT TTACTGACGA CGTTCAAAGT	1140
ATTTTTTAA AAATATTTTT TTTGTAACAA CTACTATTT TATTTATTT TTTACCTTT	1200
TATATTAAA ACATAATATA ATTTAATACT CCATCTGTT CATATTAAGT GTCATTGTAA	1260

CATTATTTT TTGTTACAAA AAAATTGTCA CTTTAGAATT CCAATGCAA ATTATTTTAT	1320
TTTCAGCTA AAATTAATTG CAAAGTGCAT TGATCTTATA AATAATTTA TTTATCTCAA	1380
ATGCTATATT GGTCAAAACAT GTGTAATTAA TAGAAACTTA ATTATATTTC ATTATTTTT	1440
TCTTAATCTG TGTAAAAATG TCAAAGTAAA ATTATTTAG AACGAATTG AGTAATATTT	1500
TGTTTCATTT TTAAAGAT ATCGAATATG AAATAACACA ATTATATTGT ATGATGAACC	1560
TAAAAATTCA TCCTAAGAAG GTGAACGCAA GAATAAGTCA ACGTTTGAG GAAAGCTAAC	1620
TATGGCCCAA AGTCATCAA ATCTTCTTG TATTTATCAA AATCCTTACA AATTAGTTA	1680
GAGTTAATAG ACCAAACACA TGATTATCAT CATATTAGAA TATTCTAAAA AATTACTAGC	1740
GAATAATTAA AATCTTTCTT TTATTATCA AAATCCTTAT AAAAACTTAT TTATATATAC	1800
TAAAACAATT TTAATTAAAA GAAAATAAGG GACCAGGAT ACATAAAAAT ATATGTTATT	1860
TCTTAAGATA GTGATAATAT TAATATATAC CAGTCCATAT ATTATCATAA ATAATAATA	1920
TTTTTCGTAG TCCGATAATC ATTACTATAA ATTCTATAAA CCACATGTAG ATGTATATTT	1980
TATTTATATA TATATATATA AACCCCTAACG CCTTACCACT CGATAACCCT CAAAACCTTT	2040
CTTCTCGTTT CGCTAACTCA AGGCTTCGAA AAGTAAAAAA ACAATGAAG AATGTCACAC	2100
TTGTTCTTGC TATGATCCTC TTCTTAAGCT GTGTCACATC CAAAGTTACA GCAACAGAAC	2160
TAGAGTCATC AACTAACCAA GAGCTCTTCC TATCGCGGCA CTTACCTCGC TTTCACCCCCA	2220
AGCAACATTG GCCGTTCCGT GGCTCCGAA AAGCCTTCCC TGCAGGCCAC TTCCGACTAA	2280
CTCCGTTCCA TCTGCCACAG GAAGTCACCA GATGCTTGAA CGACAAGAAC GAGGTAGGTA	2340
CATGTTTAA TGATATCGCT GAGACTTTCT TCACCAGGAA AGCCGCTATT GGATCGGAAT	2400
GTTGCGCCGC GATCAAGAAC ATGAACAAAG ATTGTGAGAA GACCGTCTTT GGATCTTCC	2460
ATGACCCCTT CTTGACCGGC TATGTCAGC TACATTGCTC CACCGTTGTT GGATCTACTT	2520
CACCTCCTCC TTCACAGGCT CCTTACATG CTCTTCTTC ACAGGCTCCT TCACATGCTC	2580
CTTCACATGC TCCTTCACAG GCTCCTTAA ATGCTCTTT AAATGCTCCT TTACATGCTC	2640
CTTTACATGC TCCTTCACAG GCCCCCTCAC AGGCCCCCTC ACAGGCCCC TTACATGCTC	2700
CTTTACTGCC CCCTTCGAG GCTCCCTCAC CGGCTCAGTG ATTAGCTAT TTGATAGAAT	2760
TATTCAAGTA TTGATGTCCT AGGGAGTTT AGTTTTTTTC TTGTTTAAA ATTTGTGTT	2820
TATTTGAGA AAACCGTCTT TGGATTTAA CTT	2853

CLAIMS

1. Nucleotide sequence corresponding to all or part:
 - 5 a) of the sequence according to SEQ ID No. 3, or
 - b) of a sequence which hybridizes to the sequence according to a), or
 - c) of a sequence which has at least 80% homology with a) or b).
- 10 2. Nucleotide sequence according to Claim 1, corresponding to all or part:
 - a) of the sequence which stretches from nucleotide 1 to nucleotide 2111, preferably from nucleotide 1 to nucleotide 2084 of SEQ ID No. 3, or
 - 15 b) of a sequence which hybridizes to the sequence according to a), or
 - c) of a sequence which has at least 80% homology with a) or b).
- 20 3. Cellular expression vector, comprising a sequence according to Claim 2, placed upstream of a DNA sequence encoding a cytotoxic product.
4. Vector according to Claim 3, characterized in that the cytotoxic product is a protease and preferably a subtilisin.
- 25 5. Plant cells transformed with a vector according to Claim 3 or 4.
6. Plants comprising cells according to Claim 5.
- 30 7. Plants with gametophytic male sterility with inducible fertility, comprising a gene encoding a male-gamete-specific cytotoxic product.
8. Method for producing plants with gametophytic male sterility with inducible fertility, comprising:
 - 35 - the insertion into plants of a line A of a gene whose expression product is cytotoxic for the microspores, and

- the production of plants which do not produce male gametes.

9. Method for producing plants with gametophytic male sterility with inducible fertility according to
5 Claim 8, comprising the steps of:

- a) transformation of plants of a line A with a vector according to Claim 3 or 4,
- b) induction of the fertility of the plants obtained in a) by inhibition of the cytotoxicity of the product,
10
- c) self-fertilization of the fertile plants obtained in b),
- d) selection of the plants which do not produce male gametes, derived from c),
15
- e) multiplication of the plants obtained in d) by reproduction of steps b) and c).

10. Method for producing plants according to Claim 8 or 9, characterized in that, when the cytotoxic product is a subtilisin, the induction of the fertility
20 consists in applying to the plant an insecticide molecule of the fluorophosphate family.

11. Seeds derived from the hybrid plants obtained by crossing plants of line A, which have gametophytic male sterility with inducible fertility, according to
25 Claim 7, or as obtained by using the method according to one of Claims 8 to 10, with plants of line B of agronomic value.

12. Plants according to Claim 7, or obtained by using a method according to any one of Claims 8 to 10,
30 characterized in that they belong to the Brassicaceae family and preferably in that they are rape.

M3	M3.21	'CT'GCTATG ATTTCTTCA TAAAGTCGT CACATCCAA GTCACAGCAA CAGAACTAGA GTCATCACT AACAAAGAGC	80
M3	M3.21	'CT'CC'ATC GCGGCACTTG CCTCCGCTTC ACCCCAAGGC ACATTGGCCG 'TCTCTGGCT CGGAAAGGC CTTCCCTGCA	160
M3	M3.21	GGCCACTTCC GACCAACTCC GTTCCATCTG CCACAGGAAG TCACAGGAAC TAGGTACATG	240
M3	M3.21	TTTGTATGAT' ATCGTTGAGA CTTTCTCAC CAGGAAGGCC CTTA'TGGAT CGGAATGTTG CGCCGGGATC AAGAAGATGA	320
M3	M3.21	ACAAAGATG TGAGAAGNC GCTTCTCATG CTTTCCATGA CCCCT'UC"TG ACCGGCTATG TCAAGCTACA TTGCTCACC	400
M3	M3.21	GTTGTTGGAT CTACTTCACC TCCTCTCTCA CAGGCTCCTT TACATGCTCC TTCTTCACAG GCTCCTTCAC ATGCTCCTTC	480
M3	M3.21	ACATGGCTC' TCACAGGCTC CTTTAATGC TCTTTAAAT GCTCCTTAC ATGCTCCTT ACATGGCTCCT TCACAGGCC	560
M3	M3.21	CT'GACAGGC CCTTTCACAG GCCCCCTTAC ATGGCTCCTT ACTGCCCTCT 'TGGAGGCTC CTTCACGGGC TCAGTGA-TT	640
M3	M3.21	TAGCTATTG ATAGAATTAC TCAAGTAATG ATGGCTCCTAG GAGTTTGAGT TTTAAAGT TTGCTCTTAT	720
M3	M3.21	'T'GAGAAA CCTCTTGG ATTTAACTT CACTTGTATT TCAAGTCTTGTG ATGCTCTTAG GAGT'TTGG 'TT-TCTTG TTTAAATTT TTGCTCTTAT	800
M3	M3.21	T'TGAGAAA CCTCTTGG ATTTAACTT CACTTGTATT TCAAGTCTTGTG ATGCTCTTAG GAGT'TTGG 'TT-TCTTG TTTAAATTT TTGCTCTTAT	841

FIGURE 1

FIGURE 2

1 GGATCCCACA AAGAAAAACCG AAGAAGCAAA TGTTTCCTAC CTTCATAAAT
51 ATATATTTGT TTCAGCCTCA TCAATGTACA ACAATCCTT TAGCTCAATG
101 GTATAAATGT TGTTGTTTAG ATTTCAATAA CCCGGGTTCG AGTCATAGAC
151 TTGACACTTT TTCACACTTT TTAAAAGTGG AACGCACATA TCGCTGACGT
201 GTCGCATCAG GAGTGATGCA ACTGCTCTAT TATAATGTAG ATTTAAAAGT
251 GGAACCCACG TATCGCTGAC GTGTCGCATC AGGAGTGATG CAACTGCCAT
301 ATTATAACGT AGATTTGACG TTATTCCCTT TTAAATCTTA ATAATAATAC
351 CAGNGCTTTT ACTTATTAAT TTTGNGCATN GTTATCATGG TTTATGCNCT
401 CTTTTTTTTT GANCCGTTGA TTGGTTTATG CTTATTTGAA TGTNGCCNAC
451 GTAAGAAATG AAGAACAAATT TATATTGGA GAAAATATAA TTTAATATGT
501 TCAATATATA GAGAAAAATAT TATNCCTTGA TGTTACTGTA TGGATGCGAG
551 TAGAAGATCT TTGAATAATA TTTGAGAACT TGCCTTTCT CAAAAAGTAA
601 AATATTGAT ATGTAACCTA AGTTAACACA TGAAAATTAA AAAAAAATTAA
651 AATCAAAATA GAAAAAAACTG ATAGTGATCT ACCCTTCAAC GTTTTGAECT
701 TATTCTTGGT TCACCCCCCTA AACCTCTAAG TTCACCAAAC AATAAAATT
751 CATTATTGCA TATTCTATAT CTTTAGAAA GTGAAACAAA ATATTATCAA
801 GTTATATTAT GTTTTCAAA TAAAAAGATA AAAAAATAAT AAAAAATAAT
851 AGTAGTTACA AAAAAAAAATTAATATTT TTACCAAGCGT CANAAAAACAC
901 TAAAAACCTAA ACCCTAAATA TTAAACTTTT AGGTAAACCC TAAACCTTG
951 GATAAAATCTT AAACATTAAC CATTAAAACA CTAAACCCCTA AATCCTAAAC
1001 TCTAAACCCCT TAAGTGTAA AATGTTAGT GTTTTGATT TATAGTTAG
1051 GATTTATCCA AAGGTTTAAG GTTACCCAA GAGTTTATGG TTTAGGGATT
1101 ATGACTTAGG ATTTAGTGTGTT TTACTGACGA CGTTCAAAAGT ATTTTTAA
1151 AATATTTTT TTTGTAACAA CTACTATTT TATTTATTTT TTTACCTTT
1201 TATATTAAAA ACATAATATA ATTTAATACT CCATCTGTTT CATATTAAAGT
1251 GTCATTGTAA CATTATTTT TTGTTACAAA AATATTGTCA CTTTAGAATT

FIGURE 2 (continued)

1301 CCAATGCAAA ATTATTTAT TTTTCAGCTA AAATTAATTG CAAAGTGCAT
 1351 TGATCTTATA AATAATTTA TTTATCTCAA ATGCTATATT GGTCAAACAT
 1401 GTGTAATTAA TAGAAAACCTTA ATTATATTTTC ATTATTTTTT TCTTAATCTG
 1451 TGTAAAAATG TCAAAGTAAA ATTATTTAG AAACGAATTG AGTAATATT
 1501 TGTTTCATTT TTTAAAAGAT ATCGAATATG AAATAACACA ATTTTATTGT
 1551 ATGATGAACC TAAAAATTCA TCCTAAGAAG GTGAACGCAA GAATAAGTCA
 1601 ACGTTTGAG GAAAGCTAAC TATGGCCAA AGTCATCAAA ATCTTTCTTG
 1651 TATTTATCAA AATCCTTACA AATTTAGTTA GAGTTAATAG ACCAAACACA
 1701 TGATTATCAT CATATTAGAA TATTCTAAAA AATTACTAGC GAATAATTAA
 1751 AATCTTCCTT TTATTTATCA AAATCCTTAT AAAAACTTAT TTATATATAC
 1801 TAAAACAATT TTAATTAAAA GAAAATAAGG GACCATGGAT ACATAAAAAT
 1851 ATATGTTATT TCTTAAGATA GTGATAATAT TAATATATAC CAGTCCATAT
 1901 ATTTATCAA ATAATAATA TTTTCGTAG TCCGATAATC ATTACTATAA
 1951 ATTCAAAAA CCACATGTAG ATGTATATT TATTTATATA TATATATATA
 2001 AACCCCTAACG CCTTACCACT CGATAACCAT CAAAACCTTT CTTCTCGTT
 2051 CGCTAACTCA AGGCTTCGAA AAGTAAAAAA ACAATGAAG AATGTCACAC
 2101 TTGTTCTTGC ~~TATGATCCTC~~ TTCTTAAGCT GTGTACATC CAAAGTTACA
 2151 GCAACAGAAC TAGAGTCATC AACTAACCAA GAGCTCTTCC TATCGCGGCA
 2201 CTTACCTCGC TTTCACCCCA AGCAACATTG GCCGTTCCGT GGCTCCGGAA
 2251 AAGCCTTCCC TGCAGGCCAC TTCCGACTAA CTCCGTTCCA TCTGCCACAG
 2301 GAGTCACCA GATGCTTGAA CGACAGAACAG GAGGTAGGTA CATGTTTAA
 2351 TGATATCGCT GAGACTTCT TCACCAGGAA AGCCGCTATT GGATCGGAAT
 2401 GTTGCGCCGC GATCAAGAACG ATGACAAAG ATTGTGAGAA GACCGTCTT
 M3 TTT
 2451 GGATCTTCC ATGACCCCTT CTTGACCGGC TATGTCAAGC TACATTGCTC
 M3 GGATCTTCC ATGACCCCTT CTTGACCGGC TATGTCAAGC TACATTGCTC
 2501 CACCGTTGTT GGATCTACTT CACCTCCTCC TTCACAGGCT CCTTTACATG
 M3 CACCGTTGTT GGATCTACTT CACCTCCTCC TTCACAGGCT CCTTTACATG

2551 CTCCTTCTTC ACAGGGCTCCT TCACATGCTC CTTCACATGC TCCTTCACAG
M3 CTCCTTCTTC ACAGGGCTCCT TCACATGCTC CTTCACATGC TCCTTCACAG

2601 GCTCCTTTAA ATGCTCCTTT AAATGCTCCT TTACATGCTC CTTTACATGC
M3 GCTCCTTTAA ATGCTCCTTT AAATGCTCCT TTACATGCTC CTTTACATGC

2651 TCCTTCACAG GCCCCCTTCAC AGGGCCCCCTTC ACAGGGCCCT TTACATGCTC
M3 TCCTTCACAG GCCCCCTTCAC AGGGCCCCCTTC ACAGGGCCCT TTACATGCTC

2701 CTTTACTGCC CCCTTCGCAG GCTCCTTCAC CGGCTCAGTG ATTTAGCTAT
M3 CTTTACTGCC CCCTTCGCAG GCTCCTTCAC CGGCTCAGTG ATTTAGCTAT

2751 TTGATAGAAT TATTCAAGTA TTGATGTCCT AGGGAGTTT AGTTTTTTTC
M3 TTGATAGAAT TACTCAAGTA ATGATGCCCT AGGGAGTTG AGTTTTCTC

2801 TTGTTTTAAA ATTTTGTGTT TATTTTGAGA AAACCGTCTT TGGATTTTAA
M3 GTGTTTTAAA GTTTTGTGTT TATTTTGAGA AAACCGTCTT TGGATTTTAA

2851 CTT
M3 CTT

FIGURE 2 (continued)

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WO 99/15678

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FIGURE 3

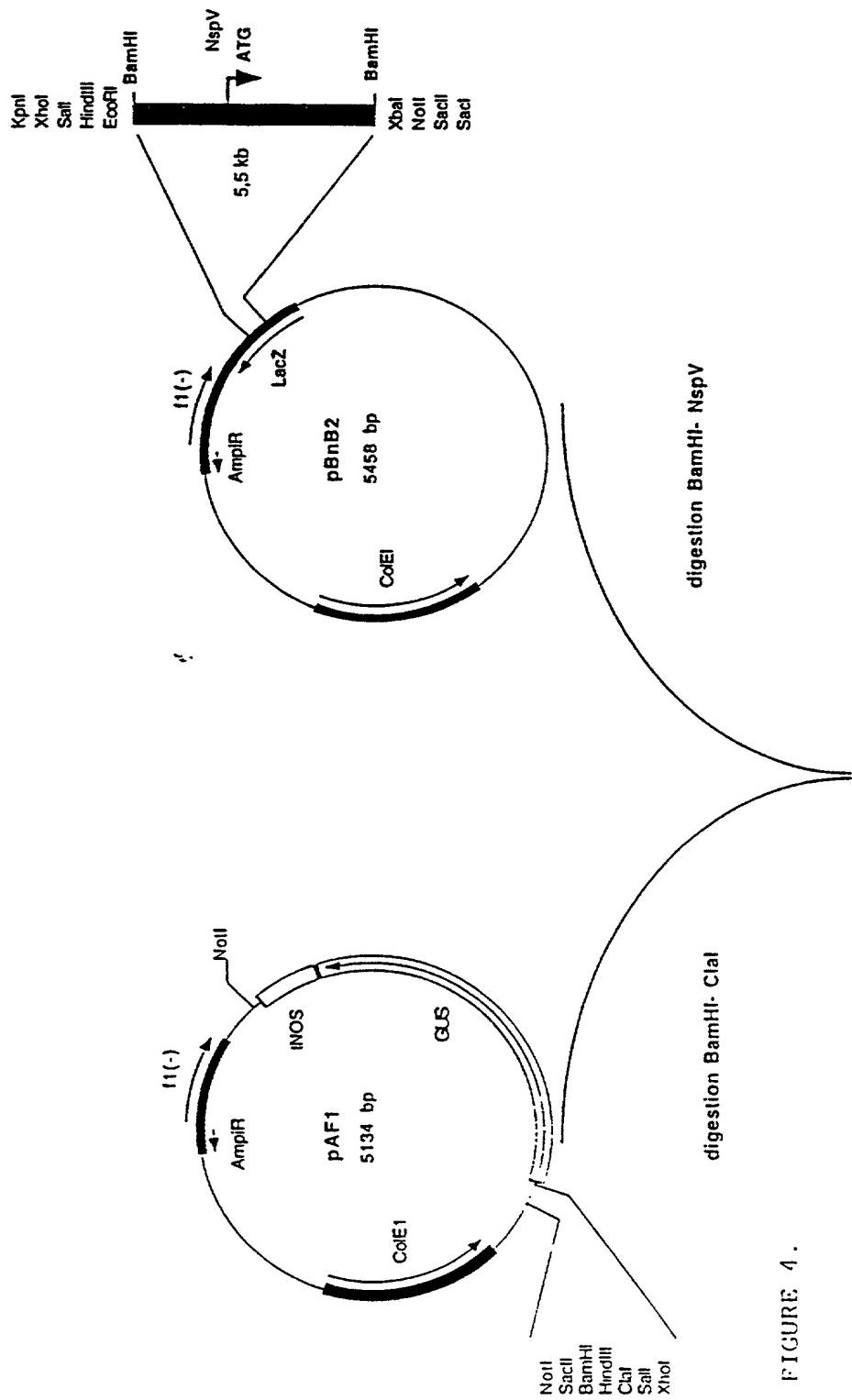


FIGURE 4 .

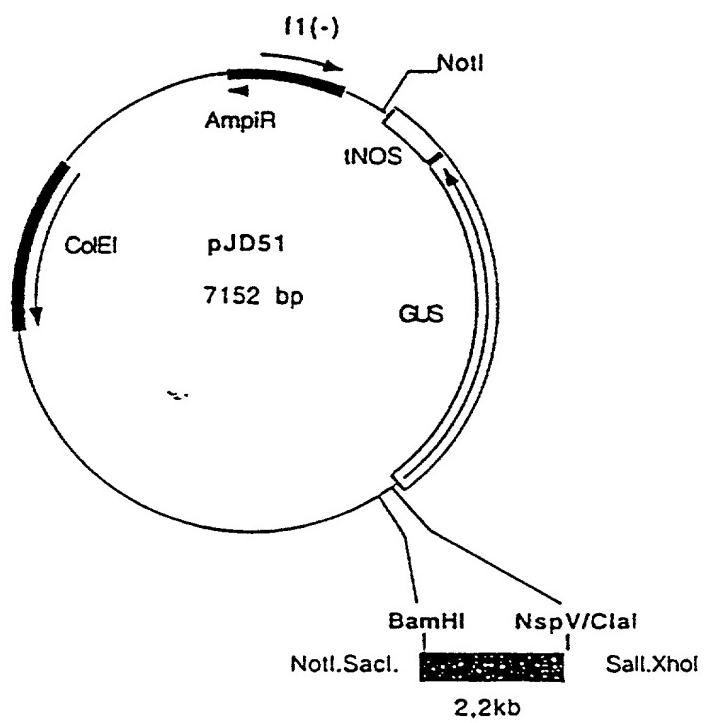


FIGURE 4 (continued)

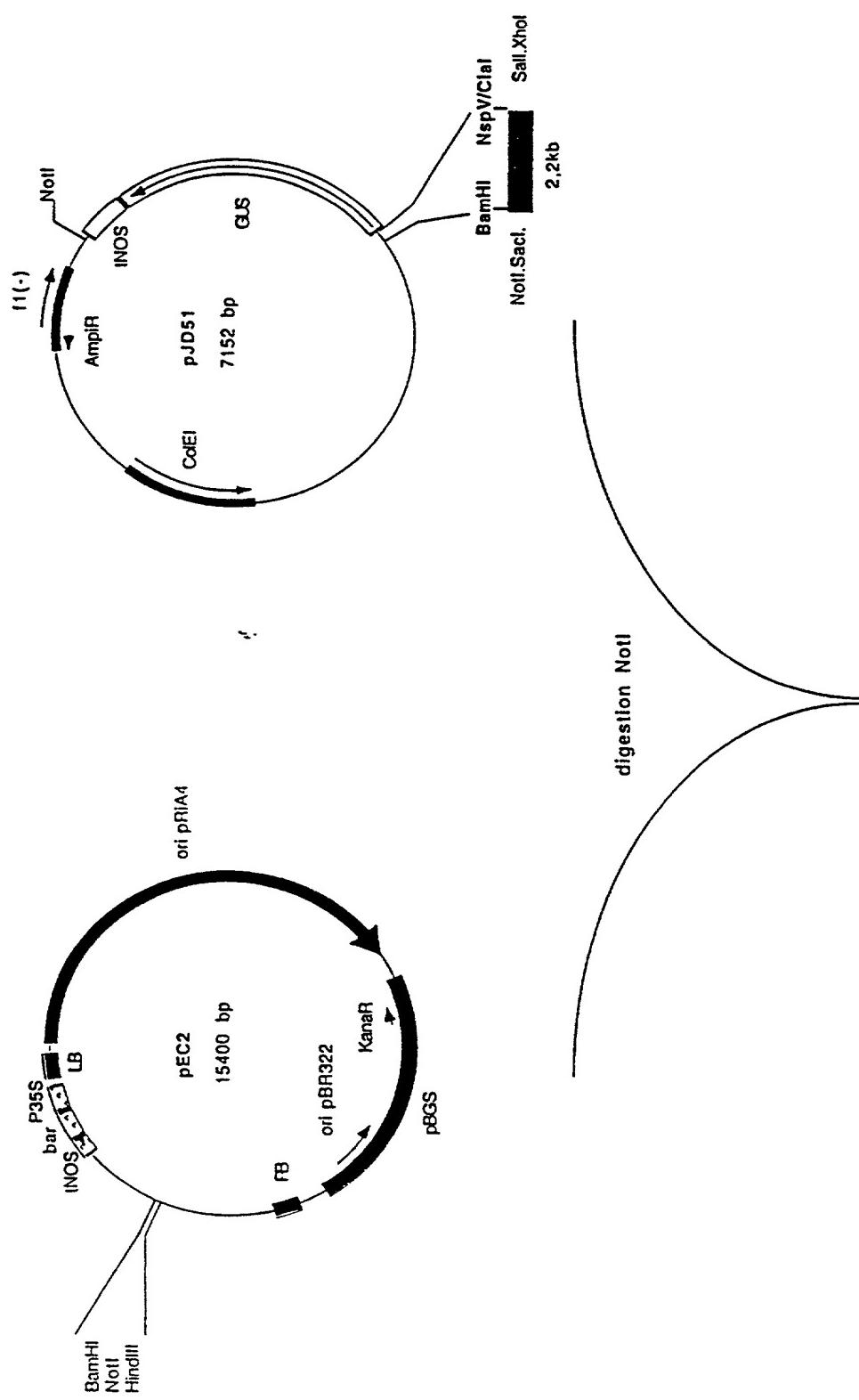


FIGURE 5.

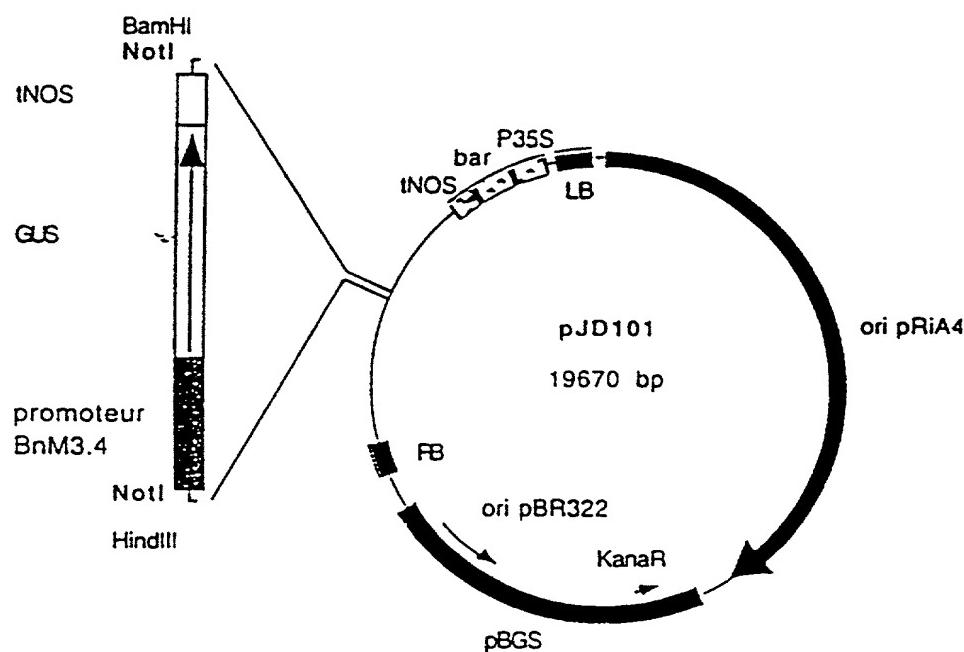


FIGURE 5 (continued)

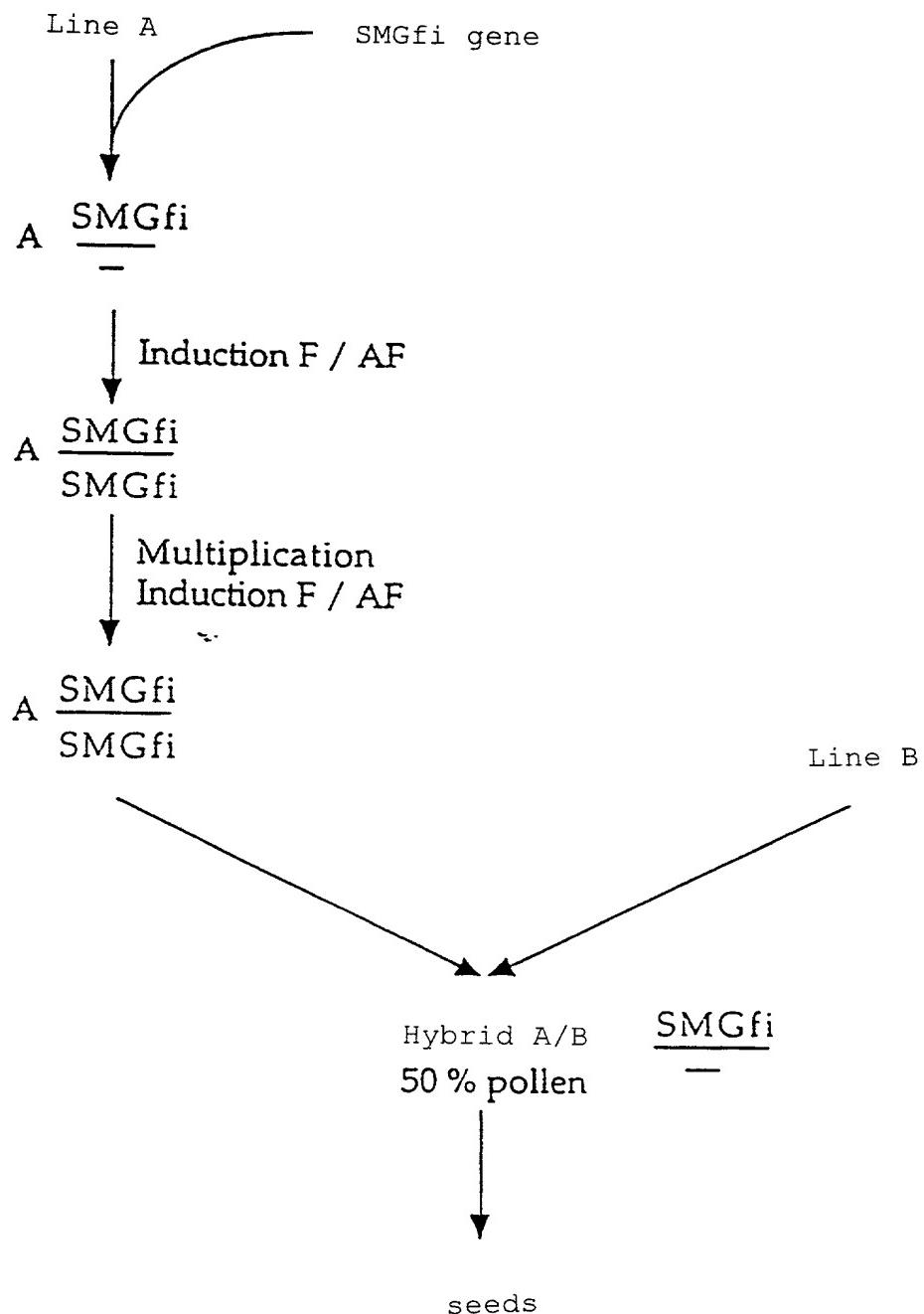


FIGURE 6

DECLARATION AND POWER OF ATTORNEY

#3

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

MICROSPORE-SPECIFIC PROMOTER AND METHOD FOR PRODUCING HYBRID PLANTS

the specification of which is attached hereto unless the following box is checked:

was filed on SEPTEMBER 23, 1998 as United States Application Number or PCT International Application Number PCT/FR98/02042 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATION(S)

NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	PRIORITY CLAIMED
97/11812	FRANCE	23/SEPTEMBER/1997	YES

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below

APPLICATION NO.	FILING DATE

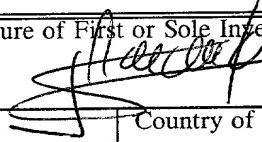
I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application

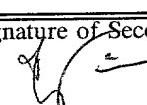
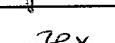
APPLICATION SERIAL NO.	FILING DATE	STATUS: PATENTED, PENDING, ABANDONED

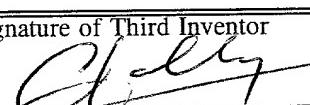
I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Stephen A. Bent, Reg. No. 29,768; David A. Blumenthal, Reg. No. 26,257; William T. Ellis, Reg. No. 26,874; John J. Feldhaus, Reg. No. 28,822; Patricia D. Granados, Reg. No. 33,683; John P. Isaacson, Reg. No. 33,715; Donald D. Jeffery, Reg. No. 19,980; Eugene M. Lee, Reg. No. 32,039; Richard Linn, Reg. No. 25,144; Peter G. Mack, Reg. No. 26,001; Brian J. McNamara, Reg. No. 32,789; Sybil Meloy, Reg. No. 22,749; George E. Quillin, Reg. No. 32,792; Colin G. Sandercock, Reg. No. 31,298; Bernhard D. Saxe, Reg. No. 28,665; Charles F. Schill, Reg. No. 27,590; Richard L. Schwaab, Reg. No. 25,479; Arthur Schwartz, Reg. No. 22,115; Harold C. Wegner, Reg. No. 25,258.

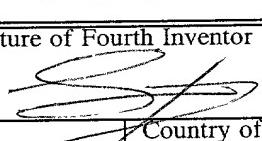
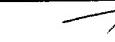
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Post Office Address <u>The same as residence</u>		

Full Name of Fourth Inventor <u>GUERCHE Philippe</u>	Signature of Fourth Inventor 	Date April 18, 2000
Residence Address <u>7, rue Marceau - 92170 Vanves - FRANCE</u>		Country of Citizenship <u>FRENCH</u>
Post Office Address <u>The same as residence</u>		

Full Name of Fifth Inventor	Signature of Fifth Inventor	Date
Residence Address	Country of Citizenship	
Post Office Address		